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Silicic and Ascorbic Acid Induced Modulations in Photosynthetic, Mineral Uptake, and Yield Attributes of Mung Bean (*Vigna radiata* L. Wilczek) under Ozone Stress

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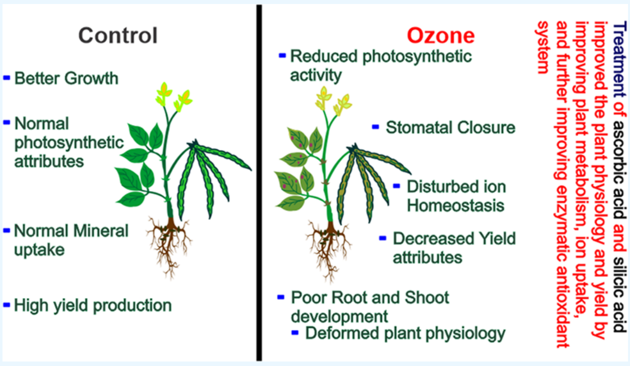

ABSTRACT: Most of the world's crop production and plant growth are anticipated to be seriously threatened by the increasing tropospheric ozone (O_3) levels. The current study demonstrates how different mung bean genotypes reacted to the elevated level of O_3 in the presence of exogenous ascorbic and silicic acid treatments. It is the first report to outline the potential protective effects of ascorbic and silicic acid applications against O_3 toxicity in 12 mung bean {*Vigna radiata* (L.) Wilken} varieties. Under controlled circumstances, the present investigation was conducted in a glass house. There were four different treatments used: control (ambient O_3 concentration of 40−45 ppb), elevated O_3 (120 ppb), elevated O_3 with silicic acid (0.1 mM), and elevated O_3 with ascorbic acid (10 mM). Three varieties, viz. NM 20-21, NM 2006, and NM 2016, showcased tolerance to O_3 toxicity. Our findings showed that ascorbic and silicic acid applications gradually increased yield characteristics such as seed yield, harvest index, days to maturity, and characteristics related to gas exchange such as transpiration rate, stomatal conductance, net photosynthetic activity, and water-use efficiency. Compared to the control, applying both growth regulators enhanced the mineral uptake across all treatments. Based on the findings of the current study, it is concluded that the subject mung bean genotypes responded to silicic acid treatment more efficiently than ascorbic acid to mitigate the harmful effects of O_3 stress.

1. INTRODUCTION

One of the major challenges for global food security is increasing crop productivity under variable climatic conditions.[1,2] O_3 pollution is the main stress factor associated with global climate change in many regions of the world.[3] Tropospheric O_3, a secondary air pollutant, harms ecosystem production and vegetation at concentrations as low as 30 ppb.[4,5] The current increase in O_3 level in the troposphere is 0.5−2% every year due to release of chemicals from manufacturing operations or industrial waste.[6,7] It is predicted that at the end of this century the tropospheric O_3 level may increase up to 18%.[8,9] Through the open stomata on leaves, it penetrates and destroys the photosynthetic system, affecting the leaf gas exchange, in addition to plant productivity, growth, and yield.[10,11] Different factors including concentration, duration of the exposure, plant development stage, conditions that affect stomatal conductance, and of course the plant species contribute toward a plant's response to fluctuating O_3 concentration in the environment.[12−14] A threshold level of 40 ppb of O_3 has been widely adopted by scientists to estimate the potential damage to plants.[15−17]

Plants have acquired avoidance or tolerance mechanisms to minimize the toxic effects of oxidizing air pollutants such as elevated O_3.[18] Avoidance mechanism is based on limited entry of O_3 into the leaf by stomatal closure.[15] The tolerance mechanism of O_3 is based on its detoxification by direct chemical reaction with apoplastic ascorbic acid, and if it enters the cellular environment, ascorbate peroxidase (APX) converts it to hydrogen peroxide (H_2O_2) enzymatically.[19,20]

Among various practices adopted to ameliorate the toxic effect of O_3, the signaling crosstalk of plant growth regulators

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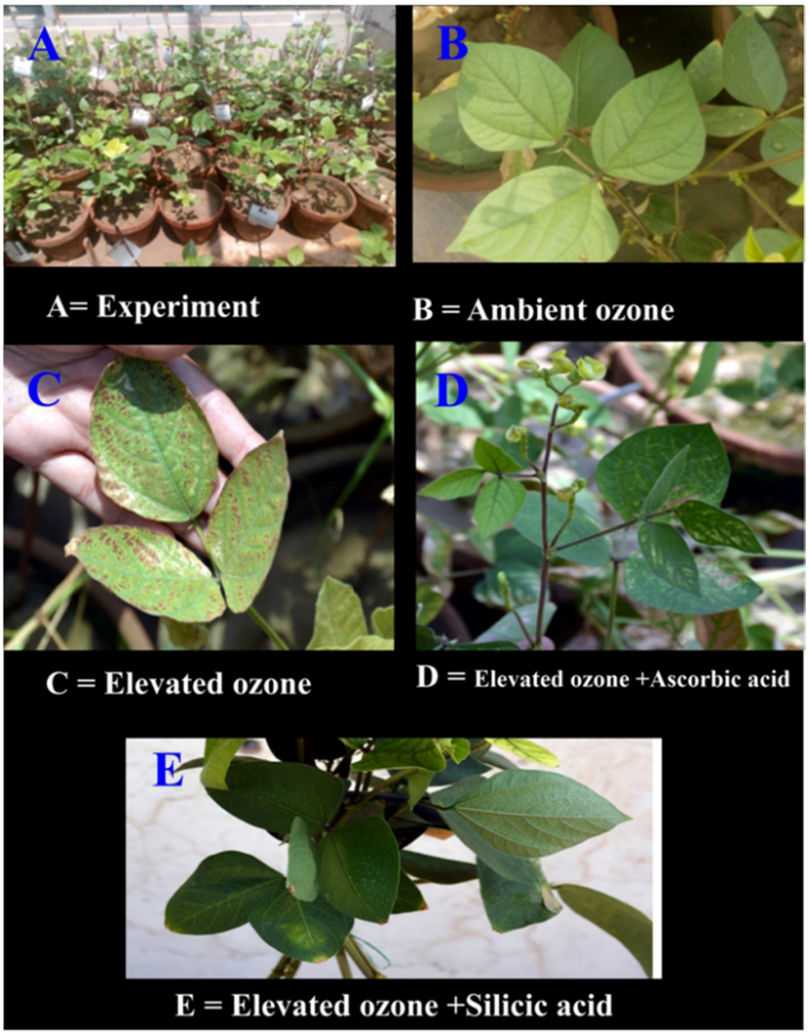

Figure 1. Experimental setup for the current study showing the experiment site (A), ambient O_3 level (B), elevated O_3 level (C), elevated O_3 and ascorbic acid (D), and elevated O_3 and silicic acid (E).

has recently attracted considerable attention from plant physiologists worldwide.[21] It is a well-known fact that plant growth regulators can protect plants against abiotic stresses like temperature,[22] heavy metals,[23,24] salt,[25] heat, O_3, and drought.[26−28] Commercially available growth promoters are widely used to improve agronomic features for high crop yield, stress tolerance, and growth promotion in many plant species.[29] There are ∼40 compounds that work as plant growth regulators, but there is very little information about the mode of action and the regulatory pathways involved.[30] There is a plethora of information regarding exogenous applications of ascorbic acid (AsA) for amelioration of various abiotic stresses in plants.[31−33] Ascorbic acid (AsA) is a reactive oxygen species (ROS) scavenger and increases the capacity of plants' oxidative defense, which results in improved growth and development under the stress condition.[34,35] Foliar spray of silicic acid has been reported to increase the yield and biomass of plants under stress. Likewise, it is also cost-effective and environment-friendly to boost plant growth.[36,37] Its deposition in the cell wall of different plant tissues makes it a barrier for toxic elements to enter in the cell.[38,39]

In the South Asian region, Pakistan, India, and Bangladesh, pulses are considered important food crops for people belonging to low-income sections. These are excellent sources of micronutrients, vegetable proteins, and essential elements, e.g., iron and nitrogen.[40,41] Thus, the demand for pulses is increasing in this area of the world. On the other hand, there was a decrease in pulse production of 36 million MT between 1998 and 2018 in the Asiatic region of the world.[42] Mung bean is one of the main but neglected kharif crops of Pakistan.[43,44] It is the most widely cultivated crop of southern Punjab in the summer season, requiring 90−120 days for maturity.[45,46] The major contribution (85%) of mung bean production is from Punjab, covering 88% of the total cultivated area of this crop.[47] Its production has been hampered mainly due to abiotic stresses including salinity, heat, ozone, and drought.[48] Selection of stress-tolerant genotypes and monitoring the effects of cheap and eco-friendly growth regulators are of prime importance for the future development of new varieties from existing germplasm. Keeping in view the importance of germplasm improvement, the present study was aimed at finding the best-performing cultivar under O_3 stress with the ultimate objective of using in a mung bean breeding program. Another objective was to explore the ameliorative impact of ascorbic and silicic acid against O_3 toxicity in mung bean cultivars.

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2. MATERIALS AND METHODS

2.1. Experimental Conditions. Faisalabad, the Manchester of Pakistan, is a famous industrial hub and is well-known for the food and textile industry. At 184 m above sea level, it is situated between longitude 73°E and latitude 31.15°N. The present study was conducted to evaluate the impact of ascorbic and silicic acid foliar application on mung bean under an elevated O_3 level. Twelve mung bean varieties, NM-28, 13-1, 19-19, 20-21, 121-25, 51, 54, 92, 98, 2006, 2011, and NM 2016, were investigated in the present study, and seeds were collected from NIAB, Faisalabad. Plants were grown in pots under controlled conditions of glass house (average temperature, i.e., 30 °C, and relative humidity, i.e., 49%) with four sets and three replications. Seeds were sterilized with 0.2% $HgCl_2$ before sowing. Three weeks after germination, 10 mM ascorbic acid and 0.1 mM silicic acid (selected from pretrial experiment) were applied as foliar spray with 0.1% Tween-20 as a surfactant (Figure 1).

2.1.1. Soil Selection. The soil was analyzed for initial physiochemical characteristics using a homogenized soil sample (pH 7.71), and the analytical results are shown in Table 1. A hydrometer was used to determine the soil texture.[49] For the basic soil organic matter calculation, the Walkley–Black methodology was used.[50] The complete and bioavailable concentrations of selected metals lead (Pb), zinc (Zn), cadmium (Cd), and iron (Fe) were assessed by digesting the soil in a particular ratio of HNO_3 and $HClO_3$[51] in addition to employing ammonium bicarbonate diethylene triamine penta acetic acid (AB-DTPA) to extract the samples.[52]

Table 1. Characteristics of Soil Used in the Experiments of the Present Study

parameters	unit	values	
textural		sandy loam	
sand	%	72	
silt	%	17	
clay	%	11	
pH		7.81	
EC	dS/m	2.01	
soluble ions			
CO_3^{2-}	$mmol_c/L$		
HCO_3^-	$mmol_c/L$	1.33	
Cl^-	$mmol_c/L$	5.85	
SO_4^{2-}[a]	$mmol_c/L$	11.41	
$Ca^{2+} + Mg^{2+}$	$mmol_c/L$	7.85	
Na^+	$mmol_c/L$	8.77	
K^+	$mmol_c/L$	0.98	
$CaCO_3$	%	1.85	
OM	%	0.97	
metal concentrations		total	available[b]
Cd	mg/kg	5.67	1.21
Cu	mg/kg	16.23	4.08
Mn	mg/kg	56.18	5.21
Fe	mg/kg	173.05	37.11

[a] By difference = TSS − (CO_3^{2-} + HCO_3^- + Cl^-). [b] AB-DTPA extractable.

2.1.2. Dispensing and Monitoring System. The O_3 gas was produced with the help of an O_3 generator (AOT-MD-500 model). Pure oxygen was used as a source of gas. This O_3 generator is an electrical device that feeds oxygen gas and converts this oxygen into O_3 gas. A system was set up for O_3 exposure in a glass house consisting of (1) an O_3 generator, (2) a UV photometry O_3 analyzer (model: 0342e), (3) an oxygen cylinder, and (4) an oxygen delivery pipe that links the O_3 generator with the oxygen cylinder. Plants were fumigated with an elevated O_3 level of 120 ppb except for the control. Continuous 4 h of O_3 monitoring was done with an O_3 analyzer (model: 0342e). The elevated O_3 level was maintained for 15 days. Normal air was considered a control treatment.

2.2. Gas Exchange Characteristics. The second youngest leaf from the top of each plant was taken for the determination of stomatal conductance (GS), transpiration rate (E), and net photosynthetic rate (Pn) by using the photosynthesis measuring-system CI-340 portable infrared gas analyzer (Analytical Development Company, Hoddesdon). The measurement of photosynthetic parameters was carried out from 9.00 am to 11.00 a.m. with standard settings as per the manufacturer's guidelines. Water-use efficiency (WUE) was calculated as (Pn/E), where Pn is the net photosynthetic rate and E is the transpiration rate.

2.3. Nutrient Analysis. *2.3.1. Digestion.* Acid-peroxide solution and oven-dried ground material (0.5 g) were both added to a 50 mL Kjeldahl flask that was heated on a dry block heater to determine mineral nutrients. The leaf Ca, P, and K contents were determined using aliquots of this solution.

2.3.2. Assessment of K^+. A flame photometer (Jenway PFP7) was used for potassium (K^+) assessment.

2.3.3. Assessment of P. Using Jackson's[53] approach, phosphorus was determined from the digested solution.

2.3.4. Calcium (Ca) and Magnesium (Mg) Determination. Determination of calcium (Ca) and magnesium (Mg) was carried out by using an atomic absorption spectrophotometer (Hitachi Z-2000).

2.3.5. Nitrogen Estimation. Micro-Kjeldhal's method[54] was used to estimate nitrogen. About 5 mL of the digested material was taken in Kjeldhal's tubes. The tubes were connected to the Kjeldhal ammonia distillation system, and each one received 5 mL of 40% NaOH. Boric acid solution (5 mL) was added to a conical flask along with a few drops of the mixed indicator. The distillation was terminated when there was about 40 mL of distillate remaining. After cooling, the distillate was titrated with 0.01 N standard H_2SO_4 until the solution turned pink. The procedure was completed with a blank.

N was estimated by the following formula

$$\%\text{ of N} = \frac{(V_2 - V_1) \times N \times 0.014}{W} \times 100$$

where V_2 is the standard H_2SO_4 volume required for the sample solution titration, V_1 is the standard H_2SO_4 volume required for the blank solution titration. N is the H_2SO_4 normality, and W is the sample weight.

2.4. Days to Maturity. The experiment site was visited daily to note the number of days taken from the emergence stage to the harvesting stage. Two plants were randomly selected and tagged from each plot to observe the number of days to maturity .

2.5. Seed Yield and Harvest Index. Total crop biomass as well as seed yield was measured following harvesting and sun-drying. The harvest index was determined as the ratio of seed yield to the total (above-ground) biological yield expressed in percentage.

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$$\text{harvest index }(\%) = \frac{\text{seed yield}}{\text{biological yield}} \times 100$$

2.6. Statistical Analysis. A split-plot design under three replications was adopted in the present study. Statistical analysis of the data was performed using Co-Stat version 6.2, Cohorts Software, 2003 (Monterey, CA), to test the significance of differences among mean values. Origin-Pro 2017 (Systat Software Inc., San Jose, CA) was employed to present data in the graphical format. Principal component analysis and correlation among the variables were performed with RStudio.

3. RESULTS

Elevated O_3 levels had a significant effect on the gas exchange properties, ions, and yield of all examined mung bean varieties, and foliar applications of growth regulators mitigated the detrimental effects of O_3 stress (see Table 2). Statistical analysis of gas exchange attributes showed that elevated O_3 significantly ($P \leq 0.001$) suppressed the gas exchange characteristics in 12 tested mung bean varieties as compared to the untreated control. Mean values showed that stomatal conductance, transpiration rate, net photosynthetic rate, and water-use efficiency were decreased in the mung bean varieties. In general, the elevated O_3 level significantly dropped ($P \leq 0.001$) the stomatal conductance of mung bean varieties up to 28%. Current results revealed that under 120 ppb O_3 level the NM-98 variety manifested a noteworthy depression (54%) in stomatal conductance as compared to the ambient O_3 level. However, foliar applied ascorbic acid (50%) and silicic acid (57%) showed maximum increases in stomatal conductance of the variety NM-98 under an elevated O_3 level (Figure 2 and Table 3).

A significant decline ($P \leq 0.001$) in the transpiration rate was observed under elevated O_3. In general, statistical analysis showed up to 74% decline. Maximum decrease was observed in two varieties NM-28 and NM-98. Foliar applications of ascorbic acid increased (62%) the transpiration rate of the variety NM-98, while silicic acid increased (58%) the rate of transpiration in the NM-28 variety.

Under an elevated O_3 condition, overall 25% less water-use efficiency was observed, whereas all 12 mung bean varieties showed differential response under an elevated O_3 level.

The var. NM-28 showed maximum reduction (39%) in water-use efficiency, while increases in water-use efficiency of 36 and 40% were observed in the variety NM-28 under foliar applications of ascorbic acid and silicic acid, respectively. Results showed that under elevated O_3 level all mung bean varieties showed 40% less net photosynthetic rate as compared to O_3 nontreated plants. About 84% maximum decrease in the net photosynthetic rate was observed in the NM-28 variety under elevated O_3, and the same was increased (65 and 76%) by foliar applications of ascorbic acid and silicic acid, respectively, in the variety NM-28.

Analysis of variance revealed that K, P, Ca, Mg, and N concentrations significantly ($P \leq 0.001$) decreased in our study in all mung bean varieties under an elevated O_3 level. Elevated O_3 levels produced a substantial drop ($P \leq 0.001$) in the phosphorus content by about 24%. However, the mung bean variety NM-98 showed a maximum decrease of 39% in phosphorus content. Likewise, ascorbic and silicic acid applications increased significantly ($P \leq 0.001$) the phospho-

Table 2. Seed Yield/Plant, Harvest Index, and Days to Maturity of 12 Varieties of Mung Bean {*V. radiata* (L.) Wilczek} Subjected to Elevated O_3 (120 ppb), Ascorbic Acid (10 mM), and Silicic Acid (0.1 mM)[a]

	seed yield/plant (g)				harvest index				days to maturity			
	T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3
NM-28	7.47 ± 0.15^{a}	4.28 ± 0.21^{b}	5.43 ± 0.20^{b}	5.62 ± 0.14^{b}	65.50 ± 4.30^{a}	30.59 ± 3.17^{c}	35.99 ± 4.26^{d}	39.17 ± 1.59^{b}	78 ± 1.19^{a}	62 ± 0.87^{d}	72 ± 0.57^{c}	75 ± 0.57^{b}
NM 13-1	7.31 ± 0.20^{a}	4.66 ± 0.26^{b}	6.26 ± 0.22^{b}	5.92 ± 0.25^{b}	41.09 ± 2.56^{b}	22.88 ± 2.11^{d}	43.90 ± 5.19^{a}	34.91 ± 3.98^{c}	83 ± 0.57^{a}	62 ± 0.87^{d}	71 ± 0.33^{b}	64 ± 0.57^{c}
NM 19-19	6.49 ± 0.14^{a}	4.88 ± 0.02^{a}	5.80 ± 0.11^{a}	6.32 ± 0.19^{a}	43.28 ± 4.23^{a}	32.82 ± 2.62^{d}	35.18 ± 3.23^{c}	38.70 ± 2.90^{b}	82 ± 0.87^{a}	70 ± 0.57^{d}	72 ± 0.66^{c}	74 ± 0.87^{b}
NM 20-21	7.46 ± 0.17^{a}	6.59 ± 0.12^{a}	6.84 ± 0.05^{a}	7.32 ± 0.14^{a}	44.96 ± 3.85^{b}	41.78 ± 4.75^{c}	44.08 ± 3.51^{b}	48.37 ± 5.50^{a}	81 ± 1.19^{a}	75 ± 0.33^{d}	76 ± 0.33^{c}	77 ± 0.87^{b}
NM 121-25	7.32 ± 0.12^{a}	5.68 ± 0.27^{a}	6.47 ± 0.15^{a}	6.27 ± 0.33^{a}	41.59 ± 1.72^{c}	29.46 ± 0.79^{d}	46.90 ± 3.75^{a}	44.33 ± 4.67^{b}	80 ± 0.33^{a}	72 ± 0.8^{c}	77 ± 0.87^{b}	73 ± 0.57^{c}
NM-51	7.11 ± 0.48^{a}	4.43 ± 0.13^{ab}	6.56 ± 0.13^{a}	6.88 ± 0.29^{a}	43.30 ± 3.06^{a}	25.95 ± 1.43^{c}	40.19 ± 4.92^{b}	42.71 ± 3.63^{a}	74 ± 0.33^{a}	57 ± 0.33^{d}	1 ± 0.87^{c}	64 ± 0.87^{b}
NM 54	6.81 ± 0.18^{a}	4.68 ± 0.21^{ab}	6.79 ± 0.16^{a}	6.91 ± 0.20^{a}	48.16 ± 1.89^{b}	34.57 ± 1.22^{d}	42.74 ± 2.09^{c}	50.72 ± 4.09^{a}	76 ± 0.87^{a}	62 ± 0.66^{c}	69 ± 1.19^{b}	70 ± 0.57^{b}
NM 92	6.29 ± 0.33^{a}	4.69 ± 0.37^{ab}	6.38 ± 0.05^{a}	7.40 ± 0.14^{a}	39.29 ± 3.56^{b}	26.75 ± 2.70^{c}	45.06 ± 4.00^{a}	46.62 ± 2.82^{a}	72 ± 0.57^{a}	61 ± 0.57^{d}	63 ± 1.14^{c}	66 ± 0.87^{b}
NM-98	7.26 ± 0.26^{a}	4.89 ± 0.24^{ab}	5.63 ± 0.19^{a}	6.77 ± 0.10^{a}	53.82 ± 5.05^{a}	26.48 ± 2.73^{d}	39.60 ± 2.31^{c}	47.28 ± 2.21^{b}	74 ± 0.87^{a}	59 ± 0.57^{d}	64 ± 1.44^{c}	66 ± 0.99^{b}
NM 2006	6.31 ± 0.13^{a}	27 ± 0.48^{a}	7.04 ± 0.34^{a}	6.08 ± 0.36^{a}	42.62 ± 3.16^{c}	35.45 ± 3.86^{d}	48.52 ± 2.46^{a}	44.92 ± 1.24^{b}	73 ± 0.87^{a}	68 ± 0.87^{d}	71 ± 0.57^{b}	69 ± 0.87^{c}
NM 2011	6.59 ± 0.18^{a}	5.03 ± 0.30^{ab}	7.17 ± 0.18^{a}	6.89 ± 0.27^{a}	46.35 ± 0.37^{b}	26.31 ± 1.22^{c}	51.01 ± 3.17^{a}	49.81 ± 5.69^{a}	72 ± 0.57^{a}	61 ± 0.87^{d}	70 ± 0.33^{b}	66 ± 0.66^{c}
NM 2016	5.41 ± 0.27^{a}	5.14 ± 0.19^{a}	5.92 ± 0.14^{a}	6.78 ± 0.21^{a}	40.57 ± 3.82^{b}	36.34 ± 4.25^{d}	38.32 ± 1.04^{c}	47.50 ± 4.61^{a}	71 ± 0.87^{a}	64 ± 1.52^{d}	66 ± 0.87^{c}	69 ± 1.14^{b}

[a] Superscript letters within rows represent significance ($p \leq 0.05$).

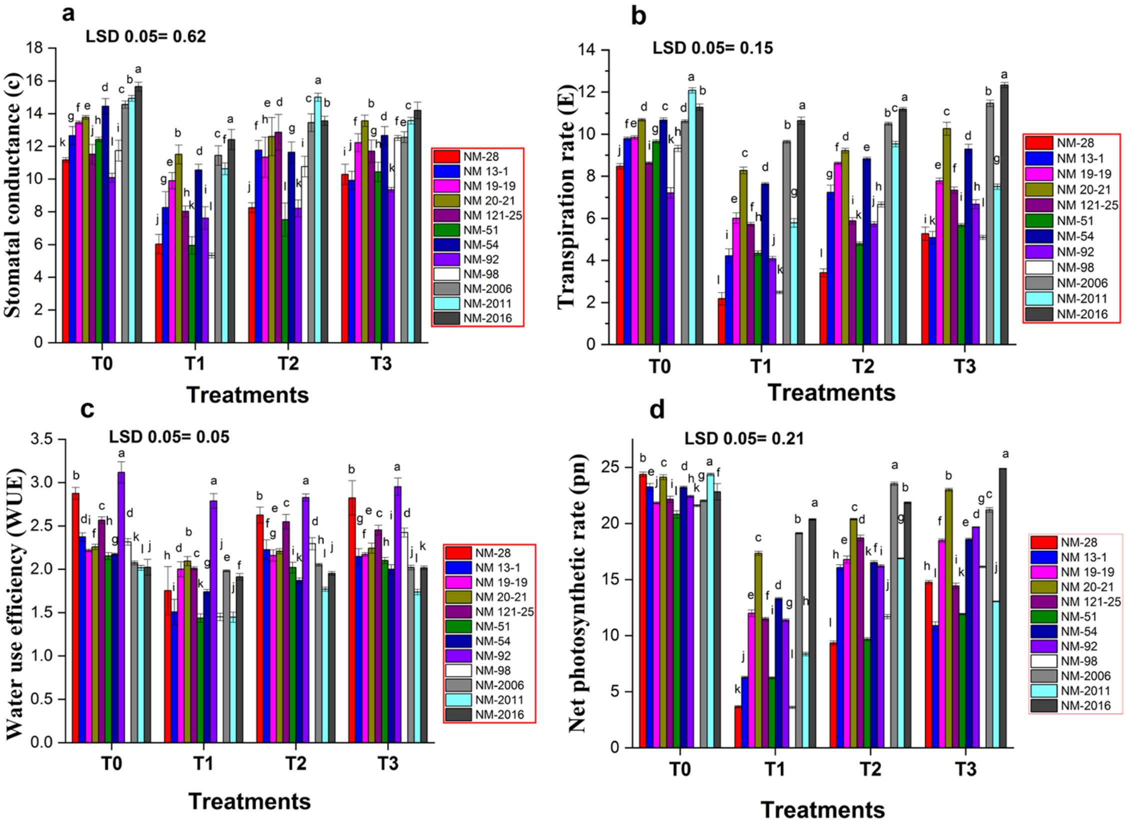

Figure 2. Under an elevated O_3 level, effect of ascorbic acid and silicic acid on stomatal conductance (a), transpiration rate (b), water-use efficiency (c), and net photosynthetic rate (d) of mung bean varieties. T0, ambient O_3 level; T1, elevated O_3 level 120 ppb; T2, elevated O_3 level + ascorbic acid; T3, elevated O_3 level + silicic acid.

Table 3. Mean-Square Values (ANOVA) for Gas Exchange Characteristics of 12 Varieties of Mung Bean {*V. radiata* (L.) Wilczek} Subjected to Elevated O_3 (120 ppb), Ascorbic Acid (10 mM), and Silicic Acid (0.1 mM)[a]

		stomatal conductance	transpiration rate	net photosynthetic rate	water-use efficiency
source	df	MS	MS	MS	MS
blocks	2	0.89 ns	0.521*	0.004 ns	0.0788*
treatment	3	104.93***	93.38***	818.51***	1.756***
main plot error	6	1.178	0.075	0.133	0.008
varieties	11	41.31***	51.11***	143.92***	1.183***
varieties × treatment	33	3.46***	3.77***	25.87***	0.092***
error	88	0.833	0.074	0.125	0.0171

[a] * Significant at the $p = 0.05$; ** Significant at the $p = 0.01$; *** Significant at the $p = 0.001$.

rus content of the mung bean variety NM-98 by 35 and 32%, respectively.

In general, calcium contents also decreased significantly ($P \leq 0.001$) in mung bean varieties up to 25% under elevated O_3 levels as compared to ambient O_3 levels. The NM-51 variety showed 48% less calcium content under elevated O_3 levels. However, the calcium content also increased by ascorbic acid (31%) in NM 13-1 and silicic acid (33%) in the NM-51 variety. Calcium accumulation was more significant ($P \leq 0.001$) in the NM-51 variety than in NM 13-1. Elevated O_3 levels produced a remarkable reduction in the magnesium content. In general, O_3-treated mung bean varieties produced 39% less magnesium content as compared to the ambient O_3 level. Of all 12 mung bean varieties, the NM-98 variety showed 60% less magnesium content, while foliar spray of ascorbic and silicic acid resulted in a profound bump (51, 56%) of magnesium in the variety NM-98 at $P \leq 0.001$.

Overall nitrogen contents decreased 37% in all mung bean varieties under elevated O_3 levels. NM-51 showed 69% less nitrogen content under elevated O_3, while there was an increase by ascorbic acid (64%) in the NM 13-1 variety and by silicic acid (63%) in the NM-51 variety. Overall potassium contents decreased notably ($P \leq 0.001$) in all tested mung bean varieties up to 18% under elevated O_3 levels as compared to O_3 nontreated mung bean varieties. The var. NM-51 showed 37% less potassium contents under an elevated O_3 level, while they were increased by 23 and 27% with application of ascorbic acid and silicic acid in this variety, respectively (Figure 3 and Table 4).

All of the tested mung bean varieties showed differential response under elevated O_3 levels. Our results showed that the overall seed yield per plant ($P \leq 0.001$), harvest index ($P \leq 0.01$), and days to maturity ($P \leq 0.001$) were significantly decreased up to 20, 25, and 15%, respectively. Elevated O_3 stress decreased maximum seed yield (42%) per plant in the

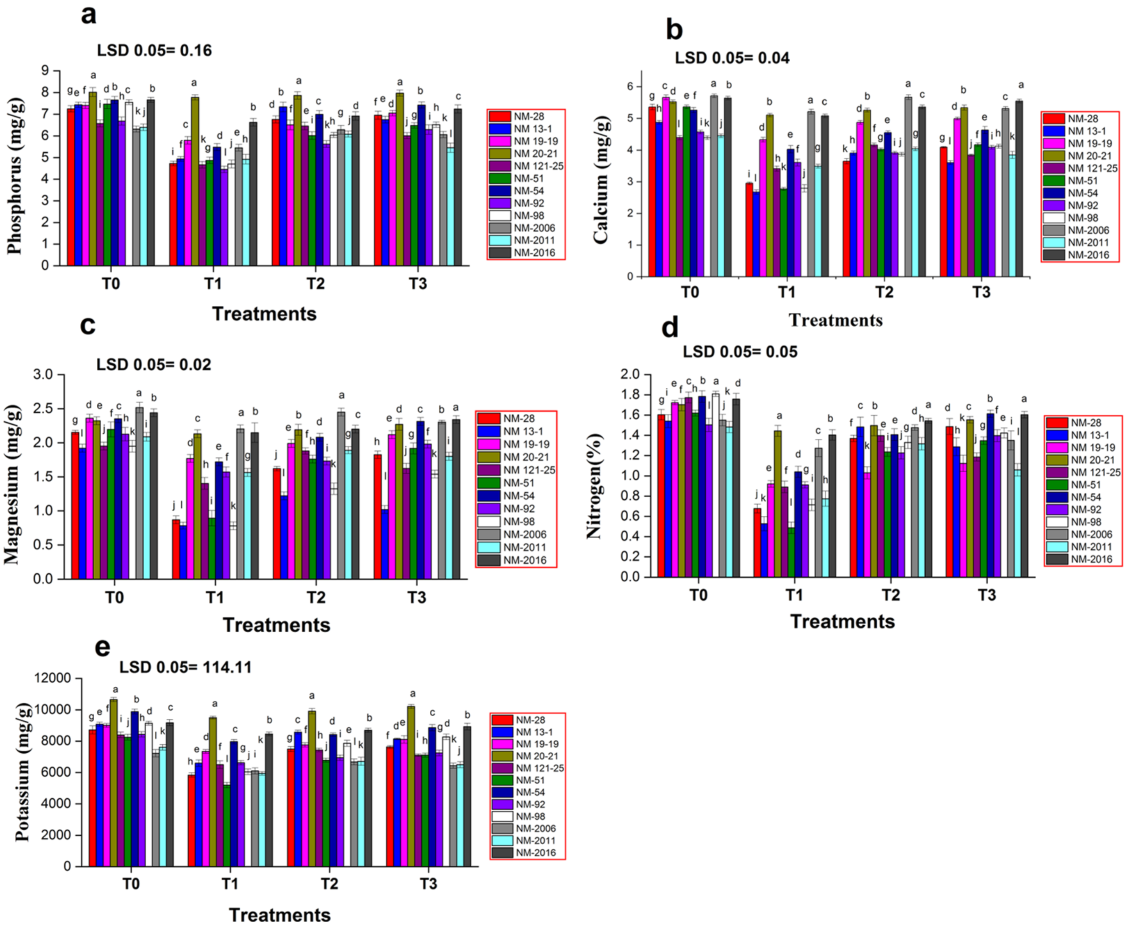

Figure 3. Under elevated O_3 level, the effect of ascorbic acid and silicic acid on phosphorus (a), calcium (b), magnesium (c), nitrogen (d), and potassium (e) contents of mung bean varieties. T0, ambient O_3 level; T1, elevated O_3 level, 120 ppb; T2, elevated O_3 level + ascorbic acid; T3, elevated O_3 level + silicic acid.

Table 4. Mean-Square Values (ANOVA) for Nutrients of 12 Varieties of Mung Bean {*V. radiata* (L.) Wilczek} Subjected to Elevated O_3 (120 ppb), Ascorbic Acid (10 mM), and Silicic Acid (0.1 mM)[a]

		phosphorus	calcium	magnesium	nitrogen (%)	potassium
source	df	MS	MS	MS	MS	MS
blocks	2	0.25 ns	0.082**	0.58***	0.001 ns	1481***
treatment	3	21.47***	10.33***	3.08***	3.29***	2309***
main plot error	6	0.086	0.006	0.001	0.01	3914
varieties	11	4.80***	5.33***	1.51***	0.24***	1253***
varieties × treatment	33	0.52***	0.34***	0.11***	0.08***	4504***
error	88	0.077	0.012	0.0018	0.01	5258

[a] * Significant at the $p = 0.05$; ** Significant at the $p = 0.01$; *** Significant at the $p = 0.001$.

NM-28 variety. However, ascorbic acid increased 30% seed yield per plant in the NM-28 variety and silicic acid application increased 33% seed yield per plant in the NM-28 variety. A maximum decrease (53%) in the harvest index by O_3 stress was observed in the NM-28 variety, and a maximum increase (45, 41%) was observed when ascorbic acid and silicic acid were applied to the NM-28 variety. A maximum reduction (24%) in days to maturity was observed in the NM 13-1 variety under O_3 stress, and maximum increases (12 and 16%) were observed in the variety NM 13-1 by ascorbic acid and silicic acid application, respectively (Tables 2 and 5).

Principal component analysis and correlation presented in Figure 4 show the correlation among nutrients, gas exchange characteristics, and yield attributes. Transpiration rate (E), calcium (Ca), magnesium (Mg), phosphorus (P), stomatal conductance (C), and net photosynthetic rate (Pn) are highly positively correlated with nitrogen (N), potassium (K), harvest index (HI), and seed yield/plant (SY). However, seed yield plant is closely positively correlated with harvest index (HI). Among the extracted components, the major contribution was from Dim 1 (67.5%) followed by Dim 2 (11.61%) with a cumulative contribution of 79.11%. It can be attributed to the fact that SY is mainly dependent on HI effectively (Figure 4).

4. DISCUSSION

Crop growth and production are primarily affected by climate variables, agronomic factors, pests, and soil nutrient availability. Any unfavorable environmental situation that impedes normal

Table 5. Mean-Square Values (ANOVA) for Yield Attributes of 12 Varieties of Mung Bean {*V. radiata* (L.) Wilczek} Subjected to Elevated O_3 (120 ppb), Ascorbic Acid (10 mM), and Silicic Acid (0.1 mM)

		seed yield/plant	harvest index	days to maturity
source	df	MS	MS	MS
blocks	2	0.58[a]	45.48 ns	17.28 ns
treatment	3	23.31[c]	1702[c]	877.58[c]
main plot error	6	0.11	38.15	10.36
varieties	11	1.50[c]	92.06[a]	213.98[c]
varieties × treatment	33	0.91[c]	101.49[b]	22.051[c]
error	88	0.27	44.43	3.962

[a] Significant at the $p = 0.05$. [b] Significant at the $p = 0.01$. [c] Significant at the $p = 0.001$.

plant development is referred to as stress. Abiotic stress negatively affects a variety of biochemistry, morphology, and physiology procedures that are directly related to plant growth and yield.[55]

Mung bean (*Vigna radiata* (L). Wilczek) is a legume that is important for farmers in South Asia due to its short duration and decent performance to cope with adverse climatic conditions.[48] Tropospheric O_3 had deleterious effects on mung bean plants, and in the current era of climate change, it is considered a very toxic air pollutant that has harmful impacts on leaf gas exchange properties.[56] Under elevated O_3 stress, transpiration rate, stomatal conductance, water-usage efficiency, and net photosynthetic rate all decreased at the same time (Figure 2). Injured leaves' stomatal conductance (GS) was observed to be slower than normal leaves.[57] The literature revealed many mechanisms by which O_3 stress may cause stomatal sluggishness or closure, reducing water-use performance. Lee et al.[58] found that a little increase in the permeability of epidermal cell membranes following exposure to O_3 and a change in osmotic pressure can modulate the turgor balance between guard and subsidiary cells.[59] Another reason for the sluggishness was that O_3 stress can trigger lower transpiration rates (E), in which leaves take longer to detect the same change in water status following petiole removal or light variations.[60] The decline in PSII photochemical efficiency (Fv/Fm) caused by the possible net loss of D1 protein of PSII reaction centers by lowering CO_2 fixation could have reduced the need for ATP and NADPH, resulting in an overdecrease of PSII.[61] It has been demonstrated that exogenous administration of plant growth regulators enhances plant growth and development under stressful environments.[62] The foliar application of silicic acid improved gas exchange properties in all mung bean varieties. In sorghum plants under water stress, Maghsoudi et al.[63] reported that foliar applied silicon plants had greater stomatal conductance, net photosynthetic rate, water-usage efficiency, and transpiration rate compared to untreated plants. In barley, corn, and wheat plants, silicon treatment against sodium chloride (NaCl) stress obtained effective results.[64] Silicon may have collected in leaves, protecting cell membranes, and increasing antioxidative enzyme activity, according to possible hypotheses.[65] Similarly, foliar treatment of ascorbic acid has been found to improve gas exchange characteristics because it is an important antioxidant that protects photochemical processes by scavenging H_2O_2, O_2^-, and OH^-, as stated in the current study and also by Ali et al.[34] and Gaafar et al.[32]

Under both stressful and nonstressed conditions, a balanced nutrient supply is essential for normal plant growth and production.[66] Since all nutrients control various physiological roles, it is recognized that N, P, K^+, Ca^{2+}, and Mg^{2+} have played an important role in cell division, cell enlargement, and differentiation.[66] The concentration of mineral nutrients like N, K^+, P, Ca^{2+}, and Mg^{2+} in all mung bean varieties was greatly decreased by elevated O_3 levels in this sample (Figure 3). As a product of altered soil chemistry and microbiology, nutrient abundance in soil under O_3 stress can be inhibited or decreased.[67] This decline in mineral content exists in rice as a result of low solubility of mineral nutrients from the root to the shoot due to reduced transpiration rates and altered membrane transporter activity. In Pb-stressed wheat plants, however, ascorbic acid application increased nutritional contents.[68] These findings may be explained by the direct effects of ascorbic acid application on cell membrane permeability, which facilitate inorganic nutrients to pass through plasma membranes, resulting in an increase in inorganic nutrient transport to metabolically important sites.[35] Furthermore, by enhancing root growth in rice and other crops, foliar application of silicic acid resulted in a substantial rise in macronutrient concentrations.[69] According

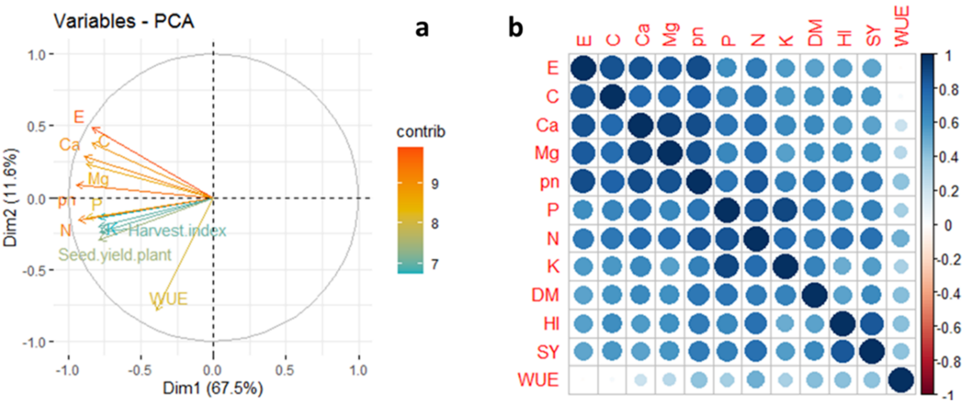

Figure 4. Principal component analysis (a) and Pearson correlation (b) showing association among nutrients, gas exchange characteristics, and yield attributes in O_3-stressed mung bean varieties treated with ascorbic acid and silicic acid. Net photosynthetic rate (Pn), stomatal conductance (c), transpiration rate (E), water-use efficiency (WUE), calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), nitrogen (N), seed yield (SY), harvest index (HI), and days to maturity (DM).

to Cuong et al.,[70] the extended root system allows for greater nutrient absorption. Greger et al.[71] found that foliar applications improved nutrient absorption in young rice plants, and they reported a similar finding. Compared to the control, foliar treatments greatly increased nutrient absorption and aggregation in the petioles of grapes.[70]

The yield attributes of all studied mung bean varieties were substantially diminished by increased O_3 stress (Table 2). Burkey et al.[72] found that increased O_3 stress lowered wheat yield attributes because the period of plant growth was shortened by reduced reproductive growth and vegetative biomass production, resulting in lower seed yield and harvest index.[73] Exogenous ascorbic acid application has been shown to improve agricultural production reduction caused by drought stress in flax, maize, and wheat.[74] Although foliar application increased nutrient uptake from the soil, it improved yield attributes without expending any energy or incurring any transit losses.[75] According to some researchers, silicon application reduced abiotic stress by improving plant reproductive growth, biomass, nutrient uptakes, and photosynthetic activity, which resulted in an increase in plant yield components.[69]

5. CONCLUSIONS

It is concluded that mung bean varieties NM 20-21, NM 2006, and NM 2016 were found to be O_3-sensitive, while NM 19-19, NM 121-25, NM 54, NM 92, and NM 2011 were categorized as O_3-tolerant. The varieties NM 19-19, NM 121-25, NM 54, NM 92, and NM 2011 were proved to be moderately sensitive on the basis of gas exchange attributes, yield production, and acquisition of mineral nutrients under an elevated O_3 level (120 ppb). However, foliar applied growth regulators such as ascorbic acid (10 mM) and silicic acid (0.1 mM) mitigated the negative effect of O_3 stress by improving the photosynthetic activity, mineral uptake, seed yield, harvest index, and days to maturity. Silicic acid showed more effective results than ascorbic acid under elevated O_3 levels. However, with regard to future prospects when O_3 pollution will be increased, these tolerant mung bean varieties and silicic acid application will be useful for mung bean growers.

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Notes

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